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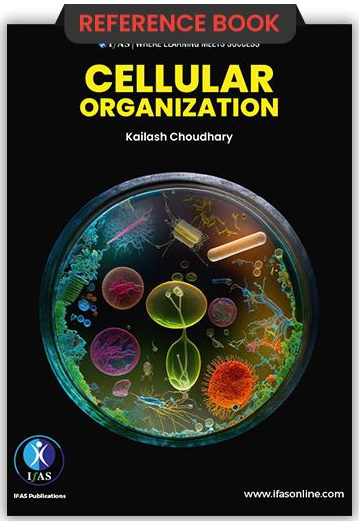
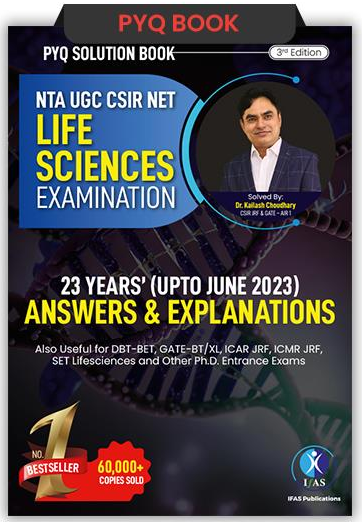
CSIR NET – Life Science

Unit 2: Cellular Organization

L4

Passive Transport

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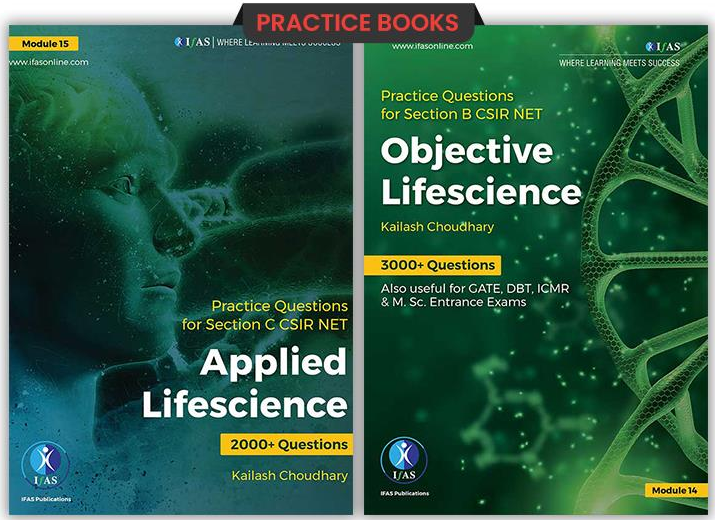

IfAS

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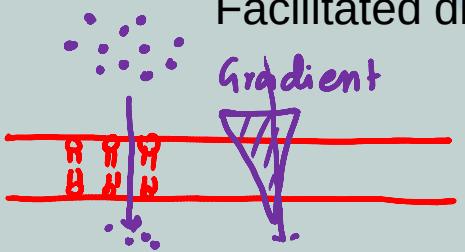
Points to be covered in this Lecture

- Diffusion, osmosis
- Ion channels and inhibitors
- Facilitated diffusion
- Transporters - GLUT, Band III, Aquaporin

Transport Across plasma membrame

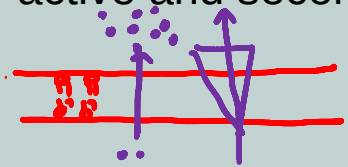
Passive Transport

- Down hill process
- Molécule Transport Along gradient K/a Passive Transport
- ATP is not required
- Example – simple diffusion (osmosis)
  Facilitated diffusion



Active Transport

- Uphill Process molecule Transport
- against gradient
- Required Energy for Transport
- Example – primary active and secondary active transport



Passive Transport

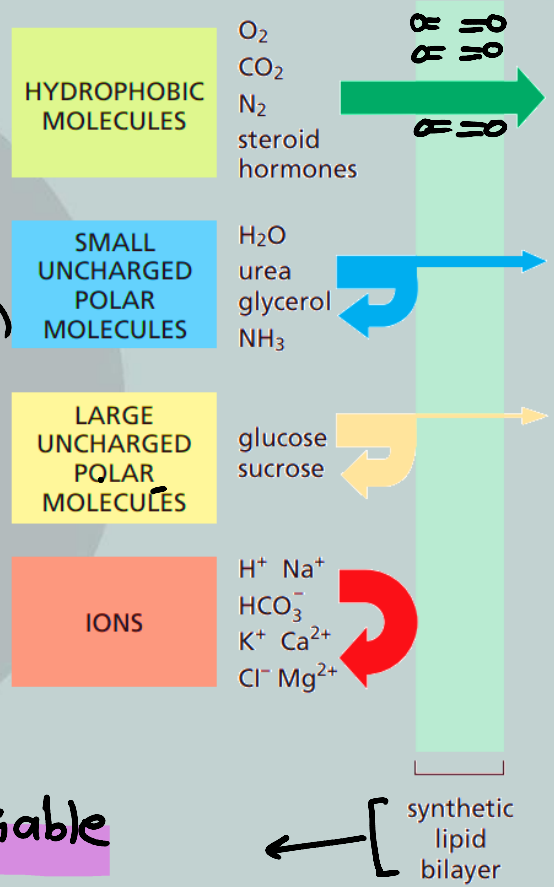
- Down hill process
- Molécule Transport Along gradient K/a Passive Transport

- Example –
  - simple diffusion (osmosis)
  - Facilitated diffusion

No Transport Needed

Transporter

Passive Transport (Along Gradient)

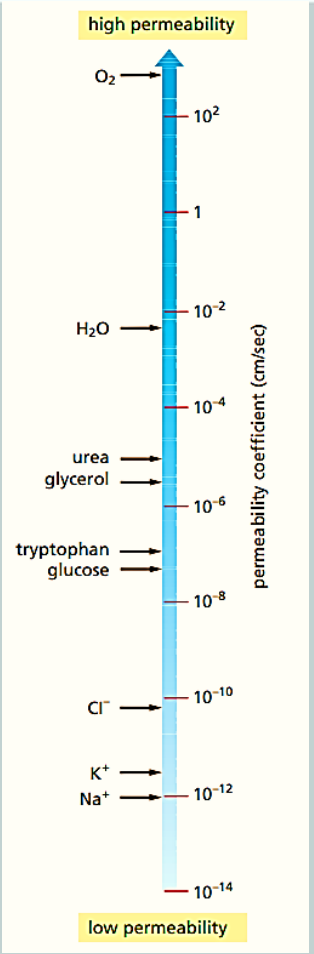


The relative permeability of a synthetic lipid bilayer to different classes of molecules.

Semipermiable

Permeability coefficients for the passage of various molecules through synthetic lipid bilayers.

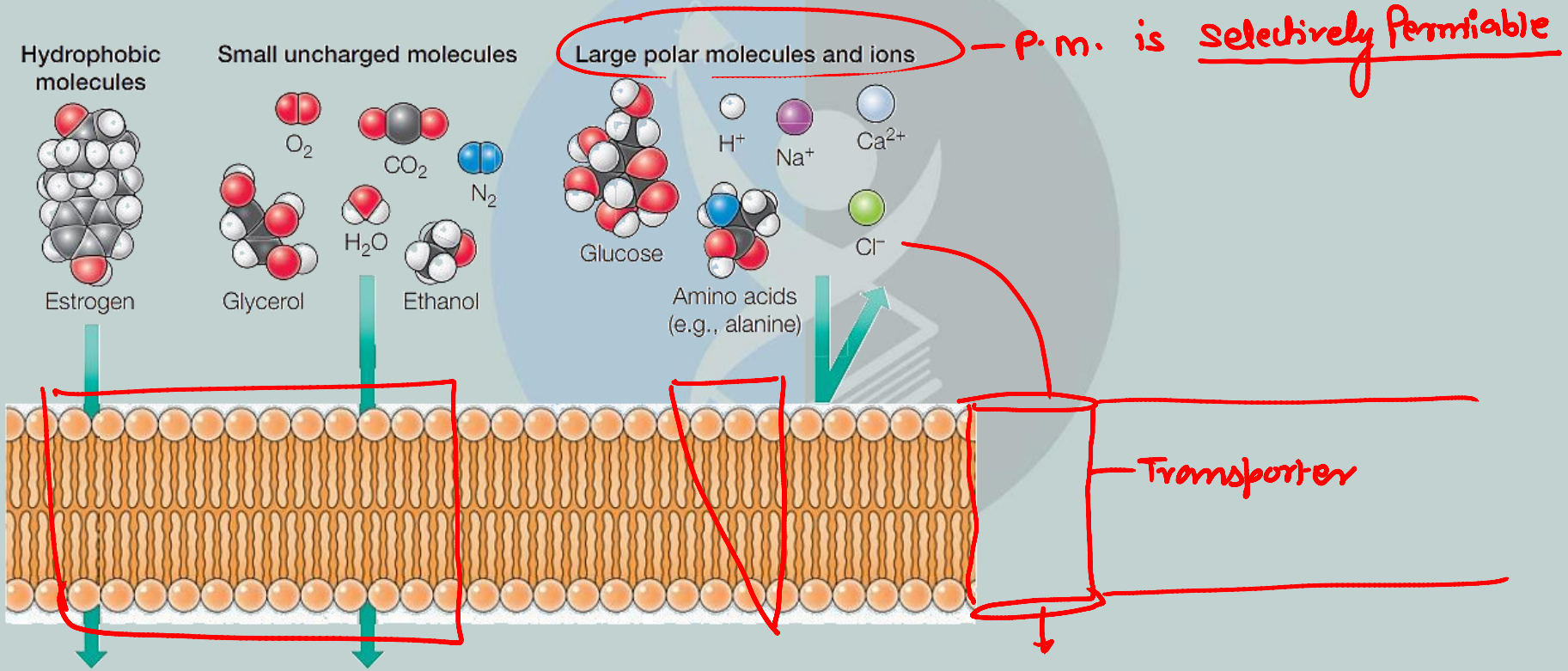
- Least – Na+
- Highest – O2



Simple diffusion

Movement of molecule along the gradient through lipid bilayer

Plasma membrane Is semi- permeable for those molecule than can pass through lipid bilayer

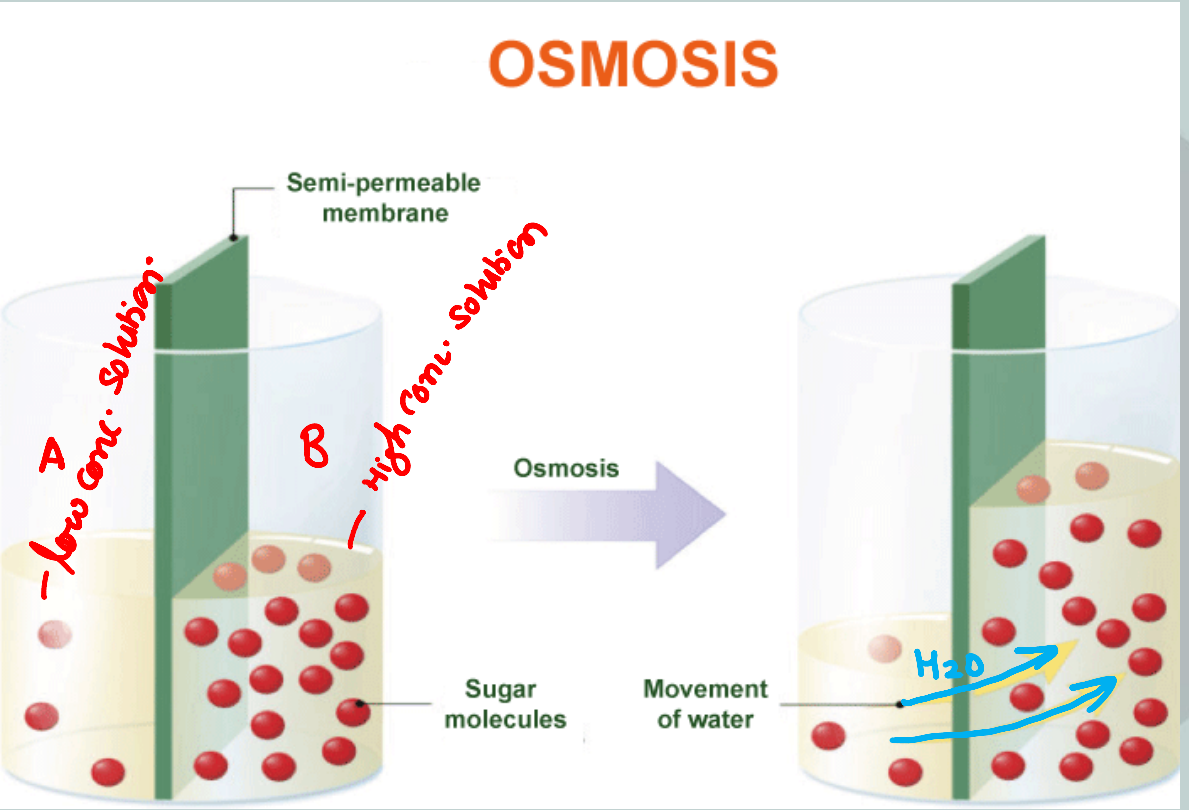


P.M. Is selectively permeable for those molecule than can not pass through lipid bilayer

Large polar molecule and charged molecule

eg.
- Glucose
- amino and

eg.
K+, Na+, Ca++
Cl-



Osmosis is the movement of solvent molecules from a solution of low concentration to a solution of high concentration, through a semipermeable membrane. There are two types of Osmosis namely Endosmosis and Exosmosis. Osmotic solutions can be isotonic, hypotonic or hypertonic.

Solute (Active osmolites)

Formula

$$\Pi = icRT$$

Π = osmotic pressure

i = van 't Hoff index

c = molar concentration of solute

R = ideal gas constant

T = temperature in Kelvin

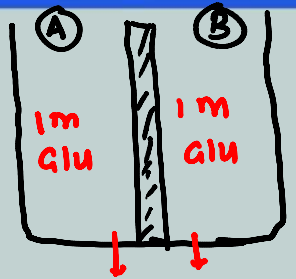
Active osmolites

- Solute that can holds water on its surface
- Actively contribute in osmotic pressure
- Solute concentration (Molar) increases = osmotic pressure increases
- Degree of ionization increases = osmotic pressure increases

- 1 M glu = 1 M glu → i = 1
- 1 M NaCl = 1 M Na+ + 1 M Cl- → i = 2

- 1M CaCl2 = ! M Ca++ + 2M cl- → i = 3

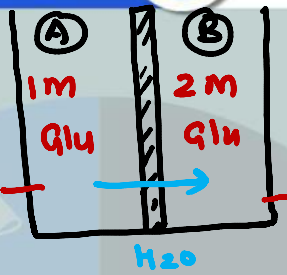
Que →



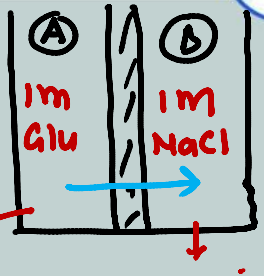
Isotonic Solution

↓

Net movement of H_2O is 0

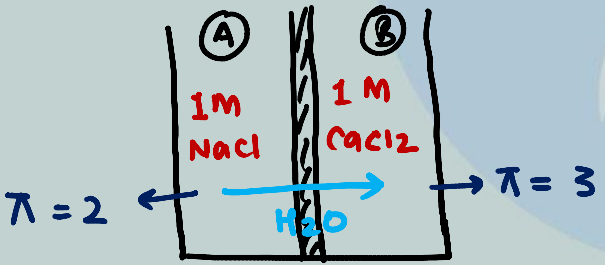


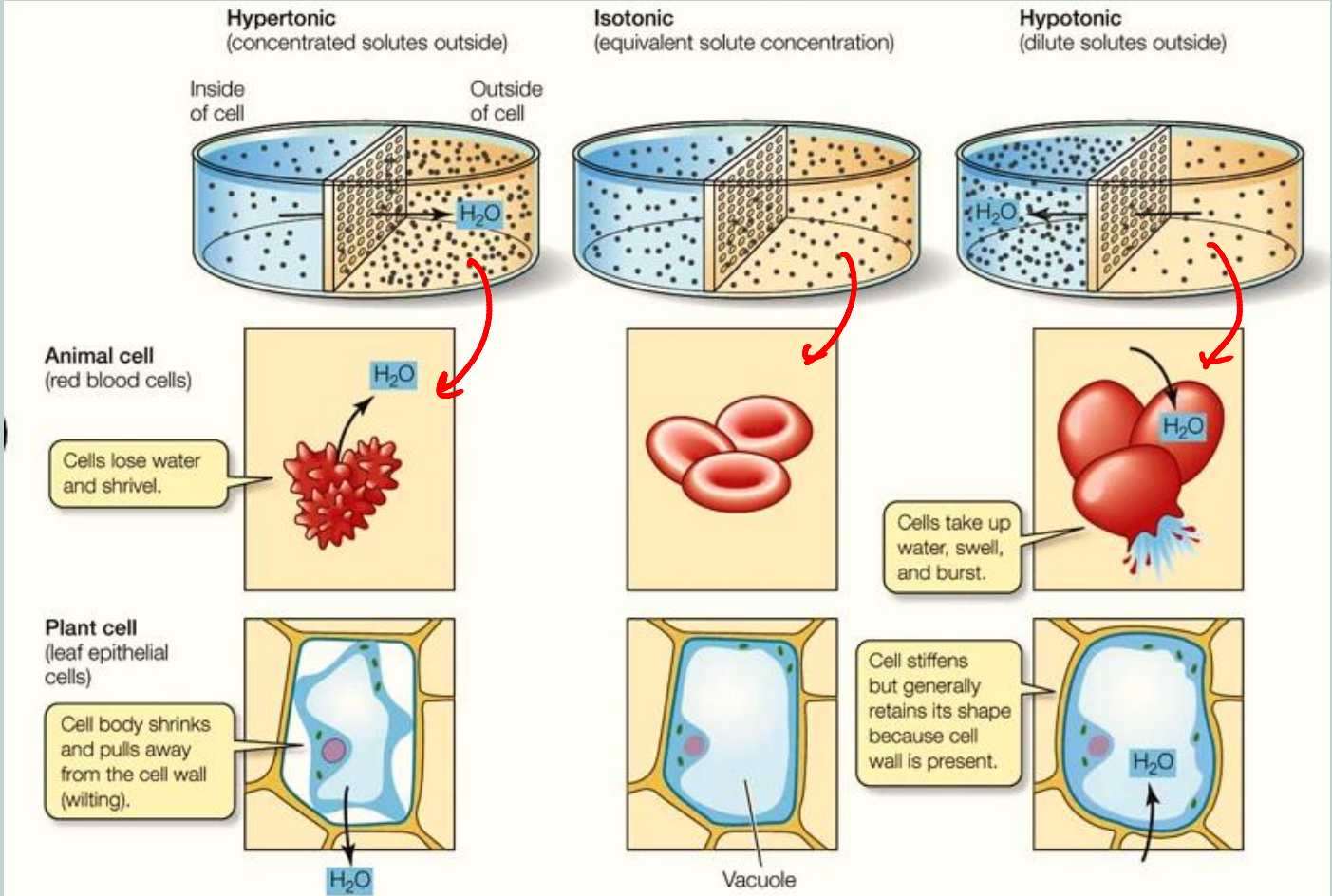
Hypo Tonic → Hypertonic

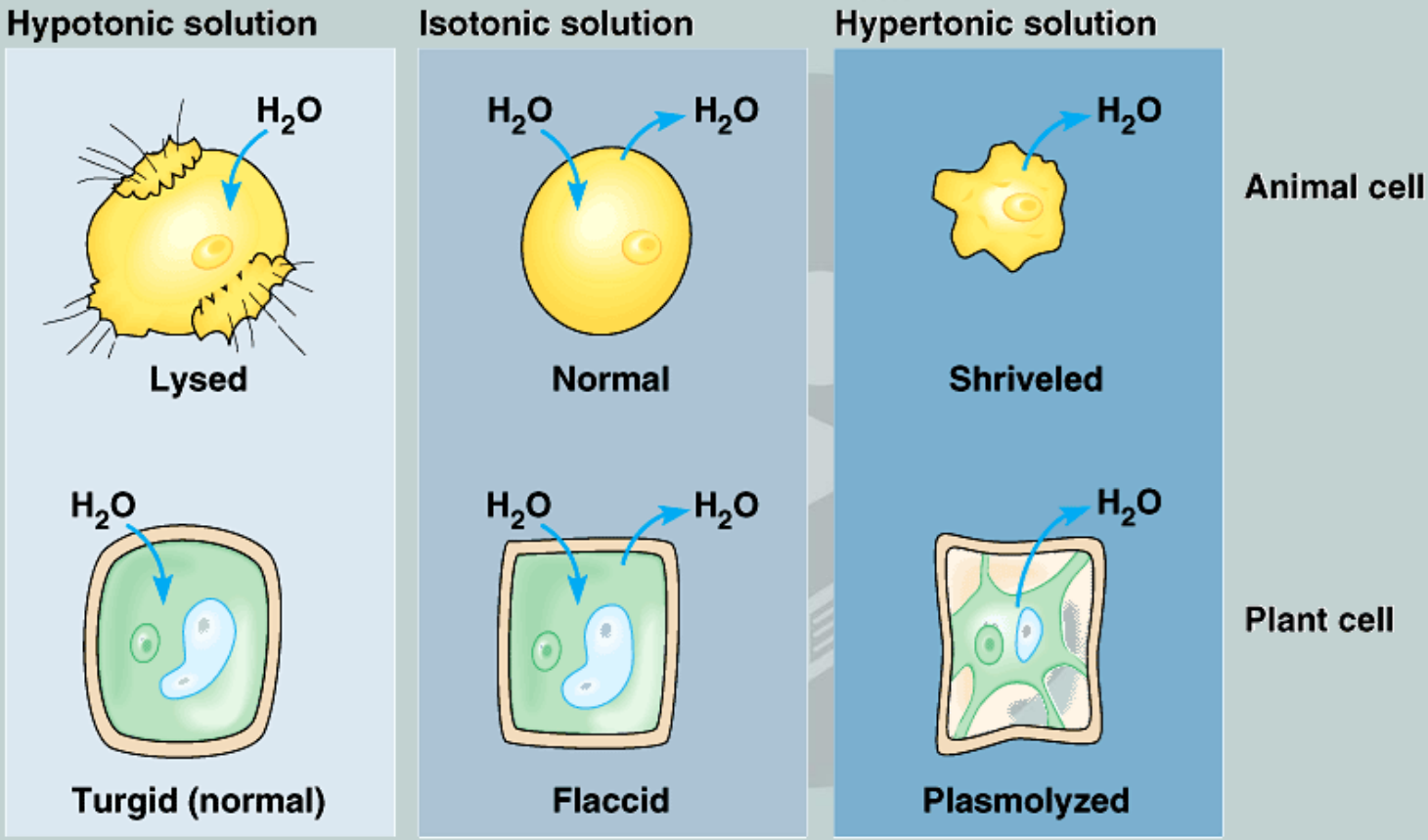


$\pi = i \cdot c$
$= 1 \times 1$
$\pi = 1$

$\pi = i \cdot c$
2×1
$\pi = 2$




Hypertonic
(concentrated solutes outside)
Isotonic
(equivalent solute concentration)
Hypotonic
(dilute solutes outside)
Inside of cell
Outside of cell
H_2O
Animal cell
(red blood cells)
Cells lose water and shrivel.
Cells take up water, swell, and burst.
Plant cell
(leaf epithelial cells)
Cell body shrinks and pulls away from the cell wall (wilting).
Cell stiffens but generally retains its shape because cell wall is present.
Vacuole


Hypotonic solution
Isotonic solution
Hypertonic solution
H_2O
Lysed
Normal
Shriveled
Animal cell
Turgid (normal)
Flaccid
Plasmolyzed
Plant cell

Diffusion	Osmosis
Solutes moves from their region of low concentration. For example diffusion of O_2 and CO_2 across alveolar-capillary membrane	It is diffusion of solvent molecules across a semi-permeable membrane. For example the movement of water molecules in a plant cell.

Rate of transport = 10^2 mol/sec

Facilitated diffusion - Transport is along gradient

Career Protein

- Carriers are integral membrane proteins that move select chemical substrates across all cellular membranes
- Career Protein Rate of Transport = 10^5 mol/sec
- Conformational Change occurs during Transport

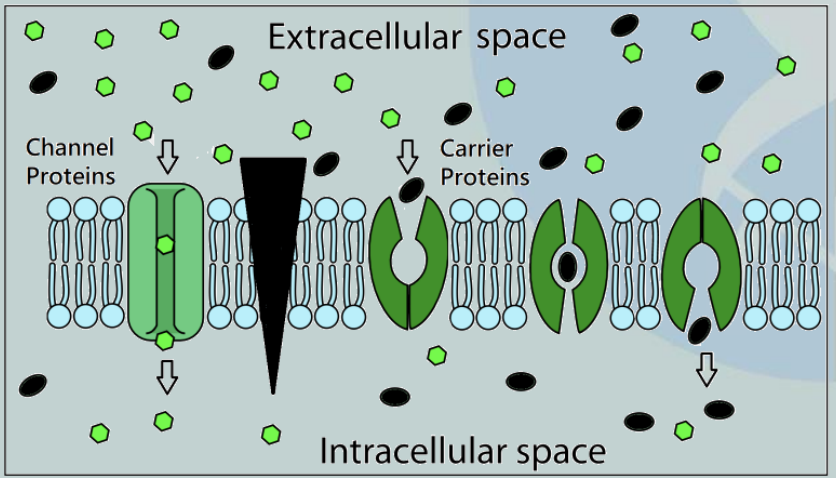
Ion Channels

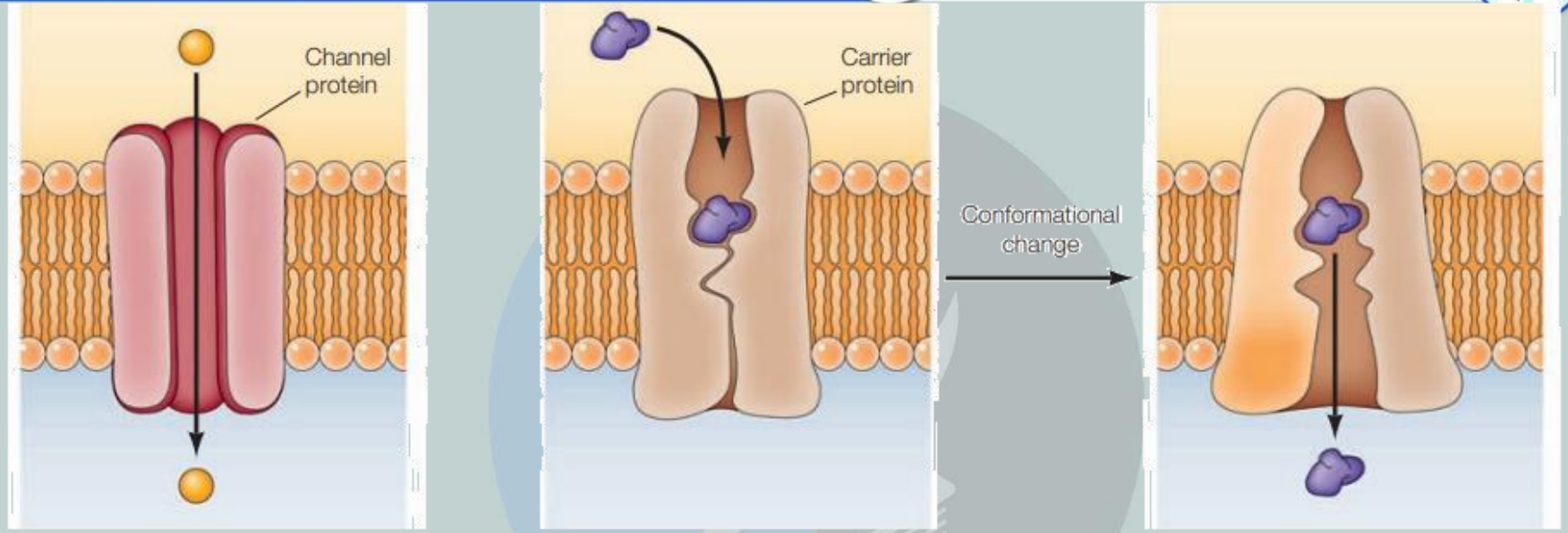
- Do not change Their Confirmation during Transport
- Rate of Transport – 10^5 -10^7 mol/sec

Ion-Channels and Carrier Proteins

[Facilitated Diffusion]
↳ Transport along Gradient

- **Ion channels** are protein pores in membrane though which specific ions are transported along gradient. There is no change in conformation and they carry out fastest mode of transport.
- **Carrier proteins** are protein which bind specifically to molecule to be transported followed by conformational change. Transport rate is slow as compare to ion channels.





Channel and carrier proteins (A) Channel proteins form open pores through which molecules of the appropriate size (e.g., ions) can cross the membrane.

→ stereospecific

(B) Carrier proteins selectively bind the small molecule to be transported and then undergo a conformational change to release the molecule on the other side of the membrane

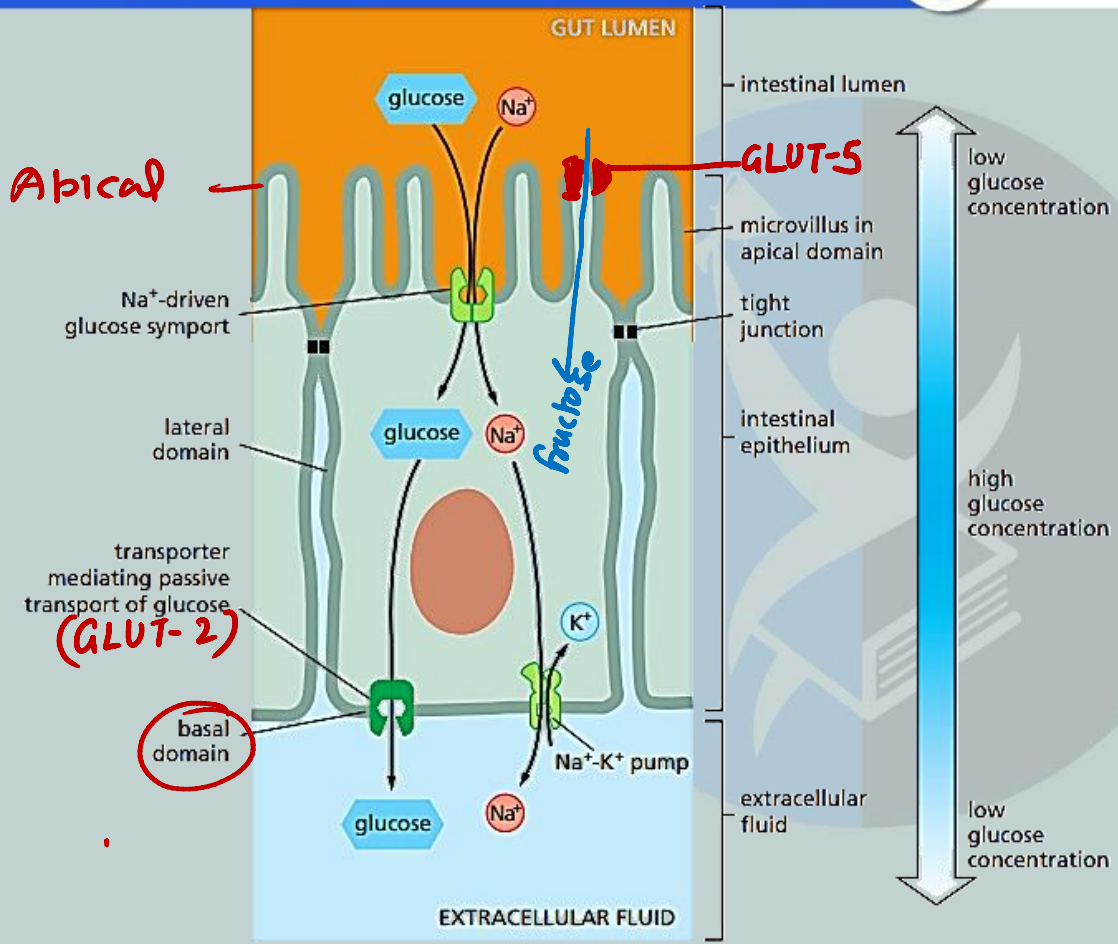
Carrier protein

- GLUT Transport
- BAND 3 Transporter
- ABC Transporter

Different Type of GLUT Transporter

GLUT 1	Erythrocytes
GLUT 2	Liver, pancreas and small intestine
GLUT 3	Brain neurons and placenta
GLUT 4	Adipose tissue, skeletal and cardiac muscles. It is insulin dependent GLUT transporter.
GLUT 5	Intestine and testis, facilitates the transport of fructose.
GLUT 12	Placenta

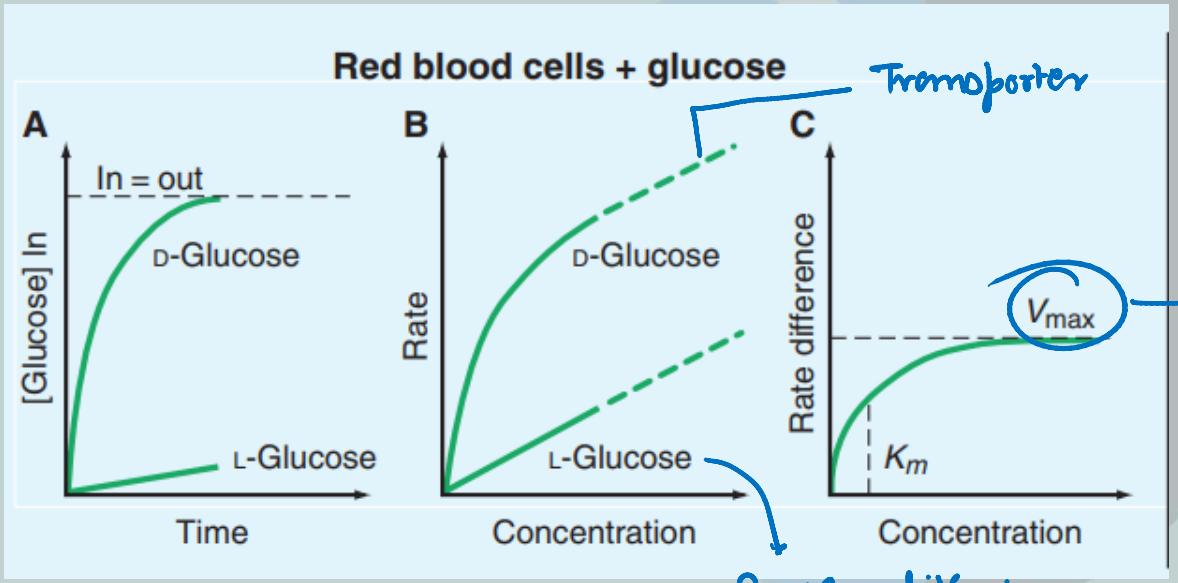
GLUT-5 → Present of the on Apical side Intestinal Epithelial cell High Affinity for fructose


GUT LUMEN
glucose
Na+
Apical
GLUT-5
Na+-driven glucose symport
lateral domain
transporter mediating passive transport of glucose
(GLUT-2)
basal domain
glucose
Na+
fructose
K+
Na+-K+ pump
glucose
Na+
EXTRACELLULAR FLUID
intestinal lumen
microvillus in apical domain
tight junction
intestinal epithelium
extracellular fluid
low glucose concentration
high glucose concentration
low glucose concentration

EXPERIMENTS THAT REVEALED THE EXISTENCE OF MEMBRANE CARRIERS

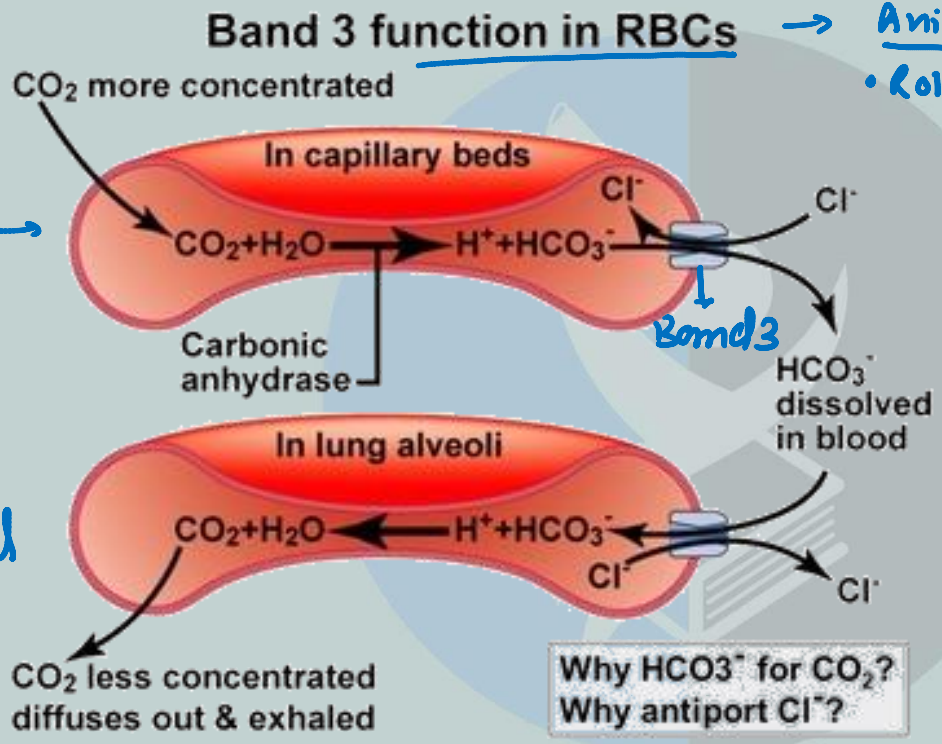
- Human red blood cells are convenient to use, because they express high concentrations of GLUT carriers.
- The time course of radioactive glucose accumulation showed that transport is stereospecific for D-glucose and that net transport stops when the concentrations of D-glucose are equal inside and out.
- Slow equilibration of L-glucose across the membrane probably represents passive diffusion across the lipid bilayer, as this rate can be predicted from the solubility of glucose in membrane lipids.
- This experiment showed that something in the membrane accelerates the rate of glucose entry, giving rise to the concept of facilitated diffusion.

EXPERIMENTS results



- A- Time course of the uptake of D- and L-glucose.
- B- Rate of uptake of D- and L-glucose as a function of extracellular concentration. Uptake of L-glucose was by diffusion across the lipid bilayer.
- C- Rate of uptake of D-glucose corrected for diffusion as a function of extracellular concentration.
- The curve is similar to the dependence of an enzyme on substrate concentration, yielding the maximum rate (Vmax) at high substrate concentration and the apparent affinity of the carrier (Km) for the substrate at half the maximal rate.

Band 3



→ Anionic Exchanger
• Role CO_2 Transport

Tissue level →

Lungs level

• Band 3 - Span - 14 times To the membrane

• Change Conformation during Transport.

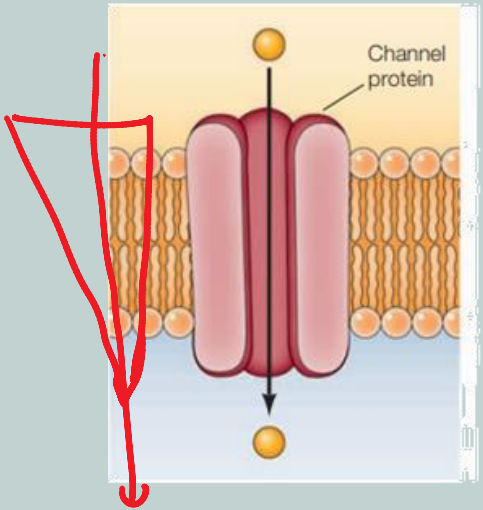
ABC transporter- Active transporter

- Contain - ATP binding Cassettes
- Confirmation change during transport
- Exception – CFTR (Cystic fibrosis trans membrane conductance regulator)

- CFTR- act as chloride channel
- Gene present on chromosome no. 7
- If mutated – cystic fibrosis occurs
- Transporter present on alveolar surface, Gastrointestinal tract (large intestine)
- Keep the surface wet

Channel [No-Conformational Change]

Non-gated/ open/ leaky

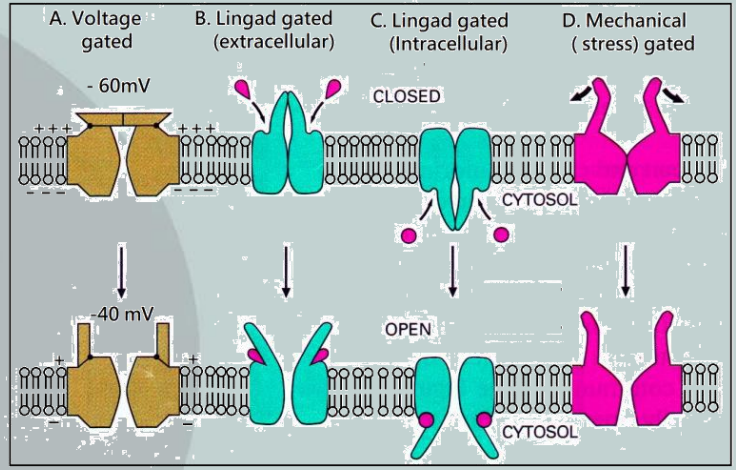


Gated [Regulated Channel]

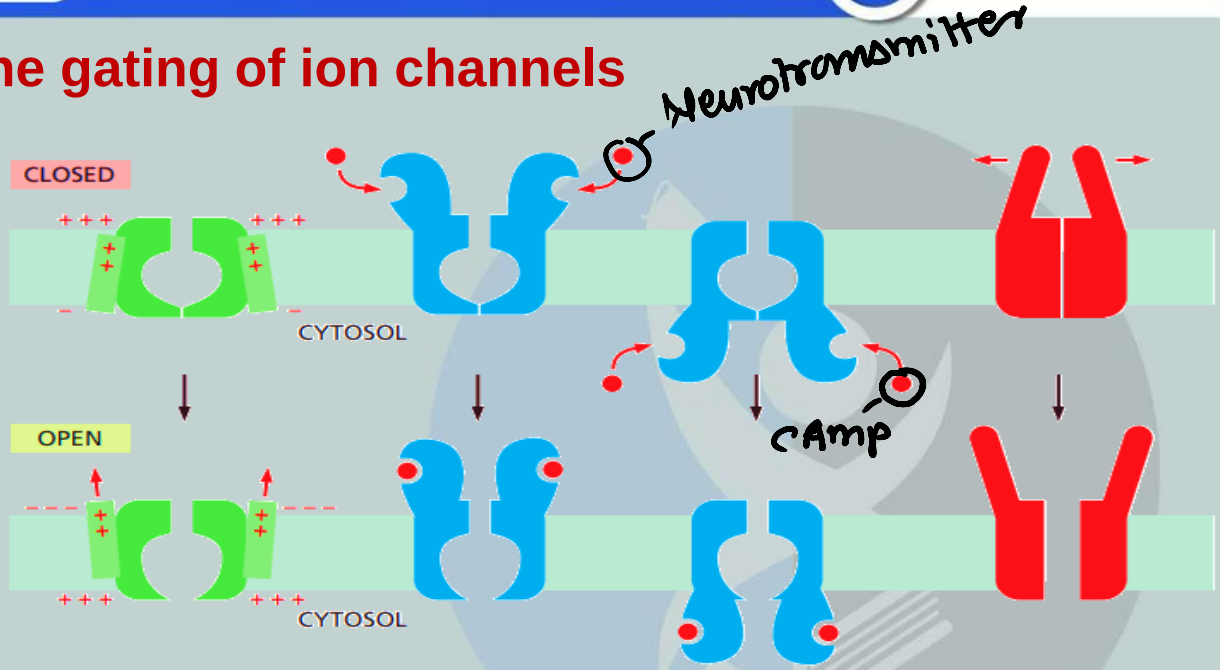
- Voltage-gated
- Ligand-gated
- Mechanically gated

Different Types of Ion Channel

- **Non-gated:** These ion channels are always open. Example include, open K^+ channel present on axon of neuron responsible for maintaining of resting potential.
- **Voltage-gated:** Open or close in response to changes in the membrane potential. Example, Na^+ along the length of axon of neuron.
- **Ligand-gated:** Activated by the binding of a ligand, such as a neurotransmitter or a hormone or secondary messenger like cAMP. Examples include: acetyl choline gated Na^+ channel
- **Mechanically gated:** These ion channels are activated by mechanical force, such as tension, stretch, or pressure. Examples include: Na^+ channel present on hair cell of cochlea in ear.



The gating of ion channels



- V.G. Na^+ channel
- V.G. K^+ Channel
- V.G. Ca^{++} Channel

eg. Nicotic acid Receptor

eg. cAMP, Ip3 Gated channel

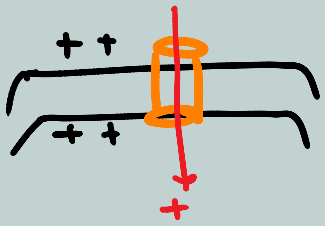
← tnr on
- Sensory Neuron
- Hair cells of Cochlea

Non-gated channels

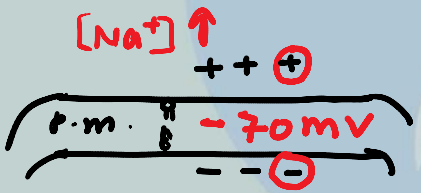
Open K+ channels – maintain resting potential

Present on plasma membrane of various cell [Neuron, Muscle]

Depolarization



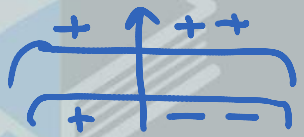
Resting potential



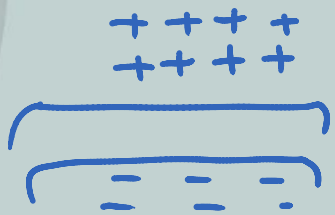
- Polarized state
- -70 mv

Resting membrane pot.

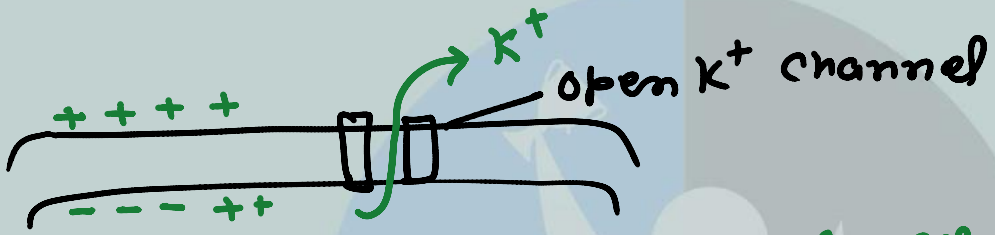
Repolarization



Hyperpolarization



Open K+ channels – maintain resting potential



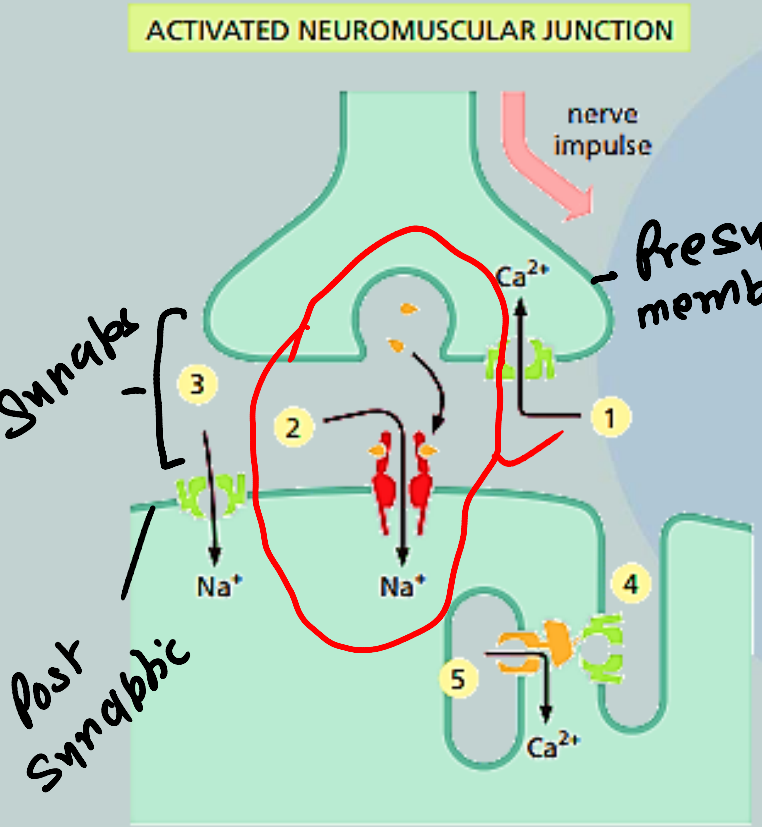
- if +ve charge rse inside of cell
- +ve charge Efflux in Form of K^+
- maintain Resting Pot.
- K^+ moves until Net movement of K^+ Reached at zero

↓

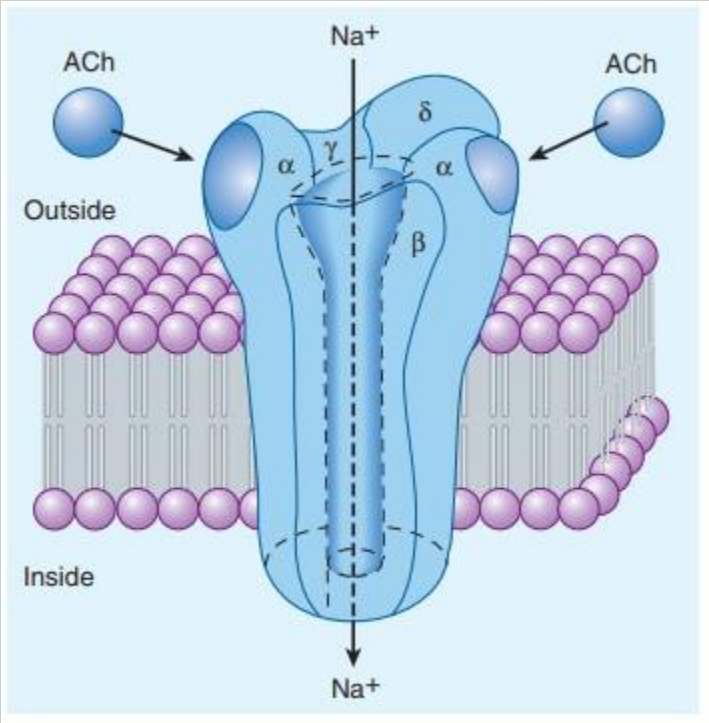
at −70 mv (Resting Pot..)

Ligand gated ion channel

neuron dendrites , neuromuscular junction- (on muscles)



- The released acetylcholine binds to acetylcholine receptors in the muscle cell plasma membrane, transiently opening the cation channels associated with them.
  Na^+ channel
- The resulting in flux of Na + causes a local membrane depolarization.
- The local depolarization opens voltage-gated Na + channels in this membrane



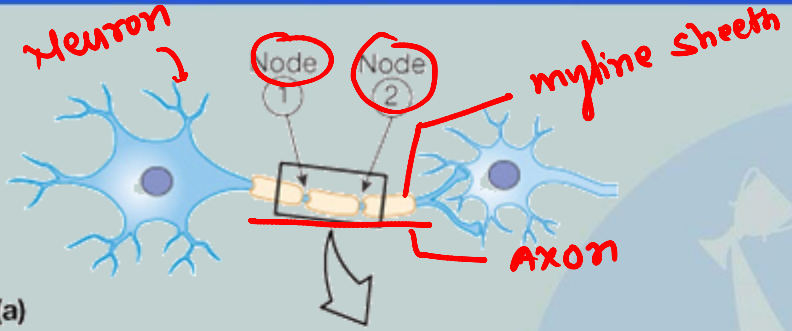
Composed of five subunits (two α, one β, one γ, and one δ), the receptor opens a central transmembrane ion channel when ACh binds to sites on the extracellular domain of its α subunits.

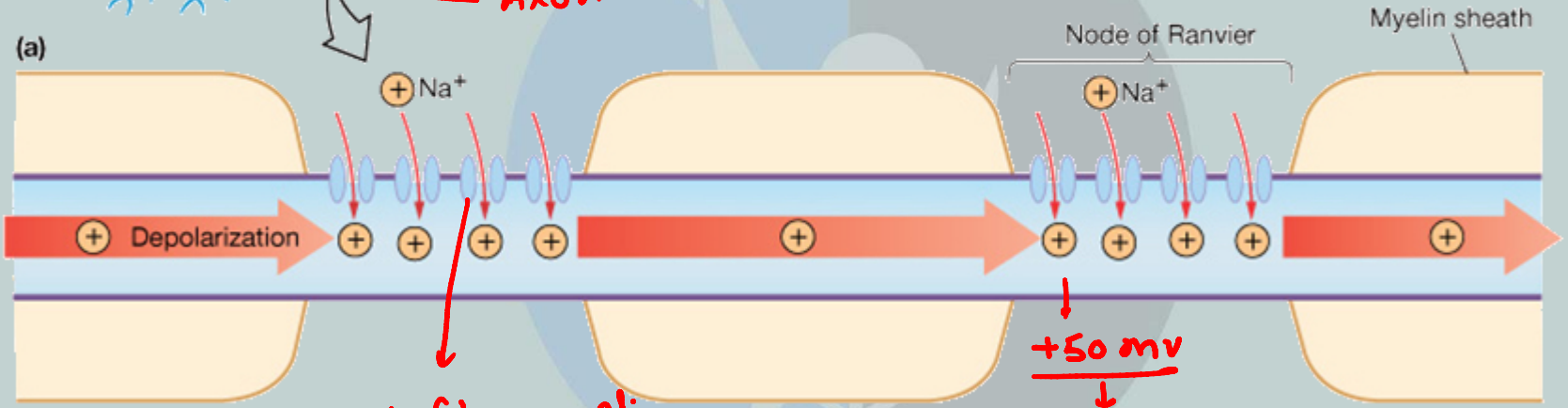
Nicotinic acid receptor/ ligand gated Na+ Channel/ Ach receptor ion channel

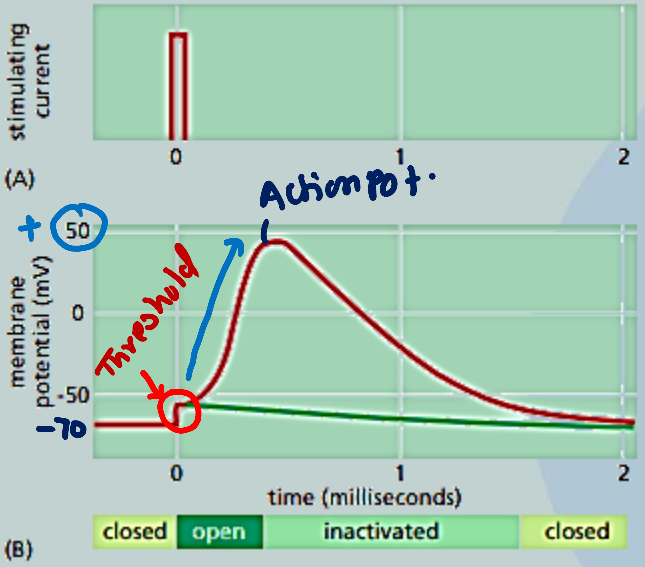
Voltage-Gated ion Channels

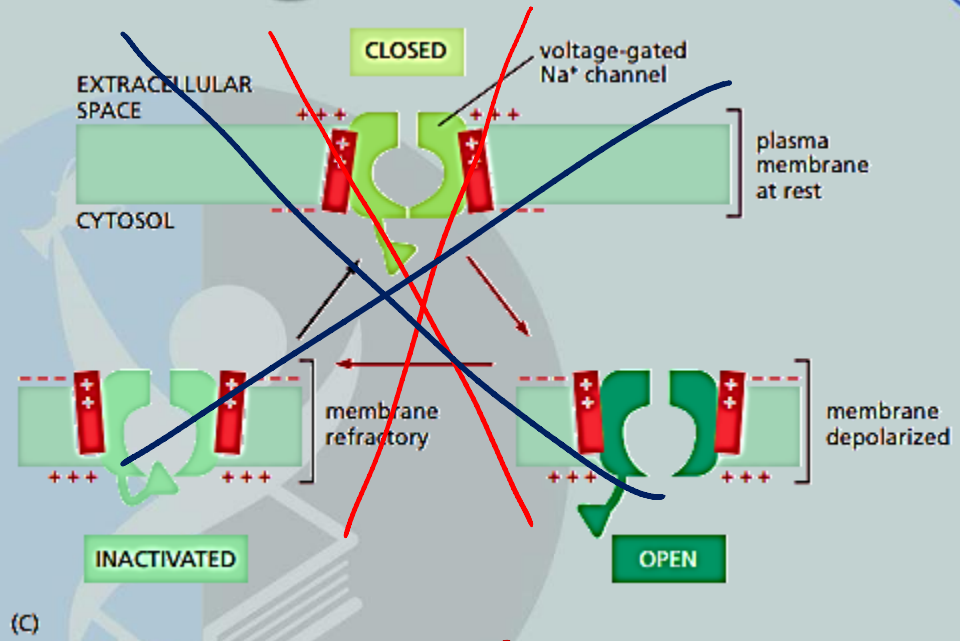
voltage-gated cation channels, which are responsible for generating the action potentials

1. In nerve and skeletal muscle cells, a stimulus that causes sufcient depolarization
2. opens the voltage-gated Na+ channels,
3. allowing a small amount of Na+ to enter the cell down its electrochemical gradient.
4. The infux of positive charge depolarizes the membrane further, thereby opening more Na+ channels, which admit more Na+ ions, causing still further depolarization.


Neuron
Node 1
Node 2
myline sheeth
Axon


(a)
Na+
Node of Ranvier
Na+
Myelin sheath
Depolarization
+50 mv
V.G. Na+ channel
Action Pot. → Conduction from 1 End to Another





The green curve shows - how the membrane potential would have simply relaxed back to the resting value after the initial depolarizing stimulus if there had been no voltage-gated Na+ channels in the membrane.

• Threshold pot.
minimum pot. (-55 mv) - Sufficient to open V.G. Na+ Channel.

Blocker of Ion-Channels

- **Bungarotoxin** blocks ligand gated Na^+ channel, thereby blocking the action of ACh at the postsynaptic membrane, inhibiting ion flow and leading to paralysis.
- **Batrachotoxin** binds to and irreversibly opens the voltage gated sodium channels of nerve cells and prevents them from closing, resulting in paralysis.
- **Tetrodotoxin (TTX),** used by puffer fish and some types of newts for defense. It blocks voltage gated sodium channels.
- **Saxitoxin** is produced by a dinoflagellate also known as "red tide". It blocks voltage-gated sodium channels.
- **Dendrotoxin** is produced by mamba snakes, blocks potassium channels.

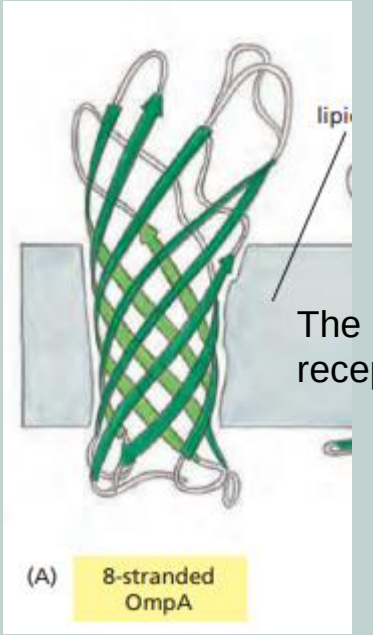
Most of channel proteins are multi subunit- alpha helix in nature
Except- **Porin channels**
Porin is made up of beta barrels

Porins are permiable to small molecule
Present on

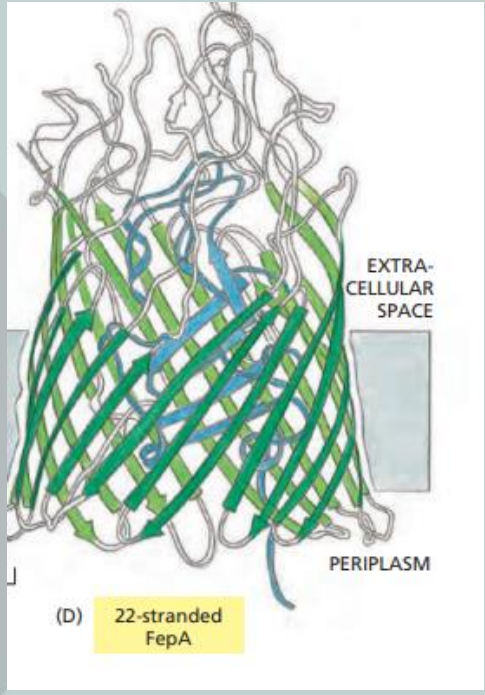
- outer mitochondial membrane

- outer chloroplast membrane
- outer bacterial membrane

- Some porins are highly selective: maltoporin, for example, preferentially allows maltose and maltose oligomers to cross the outer membrane of E. coli.



The E. coli OmpA protein serves as a receptor for a bacterial virus.



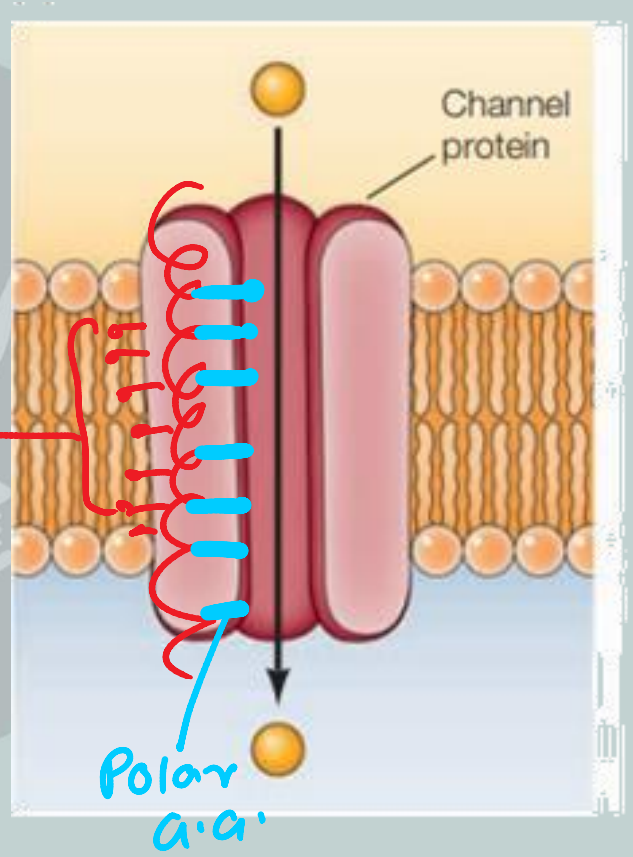
The E. coli FepA protein transports iron ions. The inside of the barrel is completely filled by a globular protein domain (shown in blue) that contains an iron-binding site.

Ion channels

- Fastest mode of Transport
- no conformational Change during transport
- made up of multiple subunit
- Amphipathic α Helix

↓

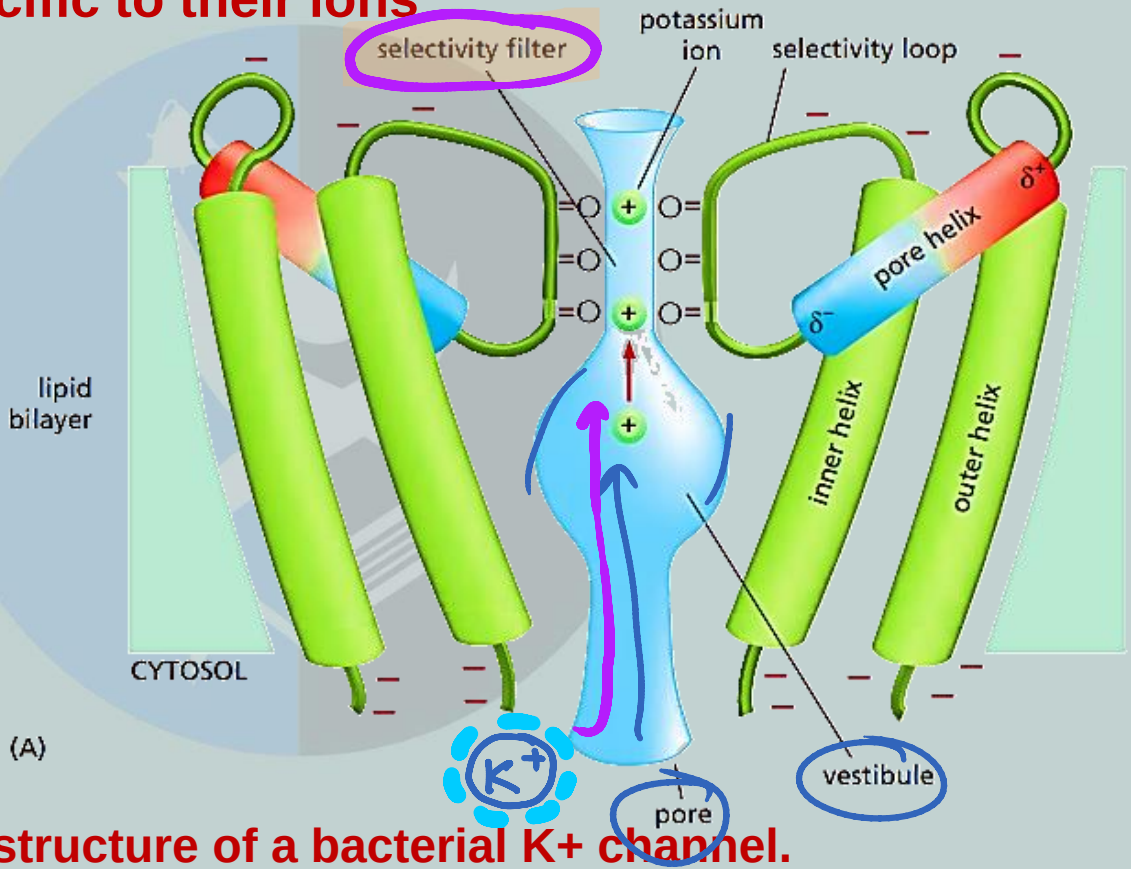
Polar & Non Polar a.a.



Channels are very specific to their ions

Reason – selectivity filter

the pore (schematically shaded in blue) opens up into a vestibule in the middle of the membrane. The pore vestibule facilitates transport by allowing the K+ ions to remain hydrated even though they are more than halfway across the membrane

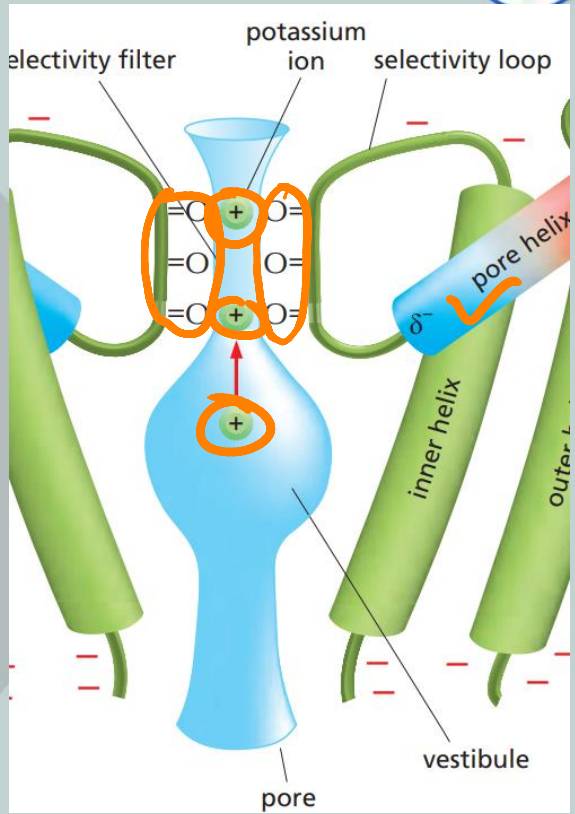


The structure of a bacterial K+ channel.

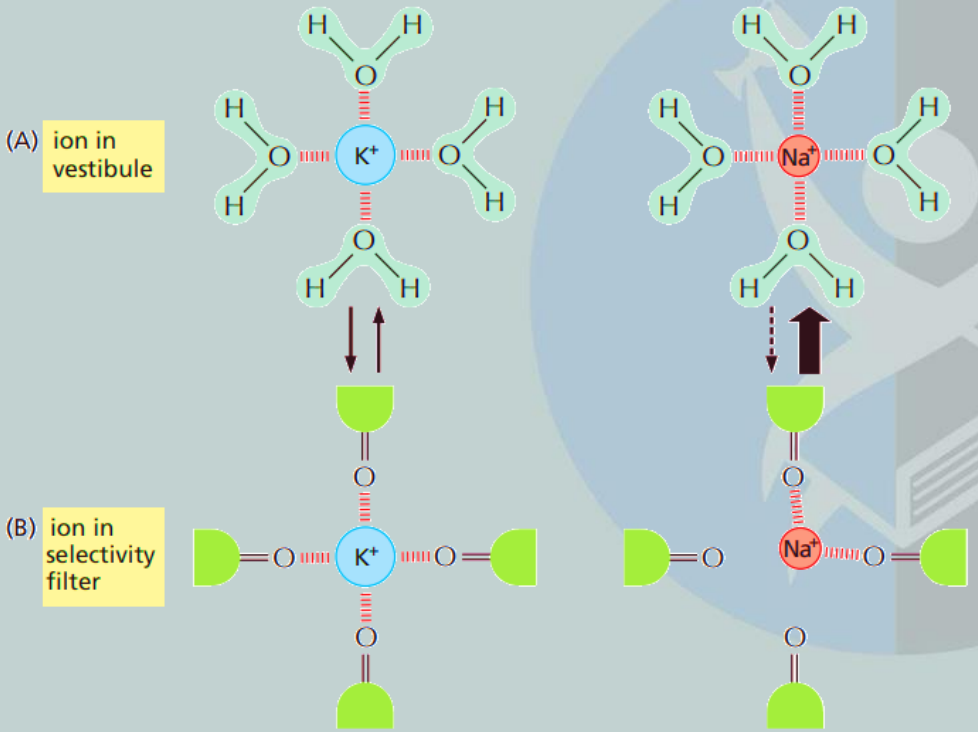
- The narrow selectivity filter of the pore links the vestibule to the outside of the cell. Carbonyl oxygens line the walls of the selectivity filter and form transient binding sites for dehydrated K+ ions.

- Two K+ ions occupy different sites in the selectivity filter, while a third K+ ion is located in the center of the vestibule, where it is stabilized by electrical interactions with the more negatively charged ends of the pore helices.

- The ends of the four short “pore helices” (only two of which are shown) point precisely toward the center of the vestibule, thereby guiding K+ ions into the selectivity filter



K+ specificity of the selectivity filter in a K+ channel



(A) in the vestibule - the ions are hydrated.

(B) in the selectivity filter of the pore- they have lost their water, and the carbonyl oxygens are placed to accommodate a dehydrated K+ ion.

- Na+ ion is too small to interact with the oxygens, it can enter the selectivity filter only at a great energetic expense.
- The filter therefore selects K+ ions with high specificity

Aquaporin – water channel

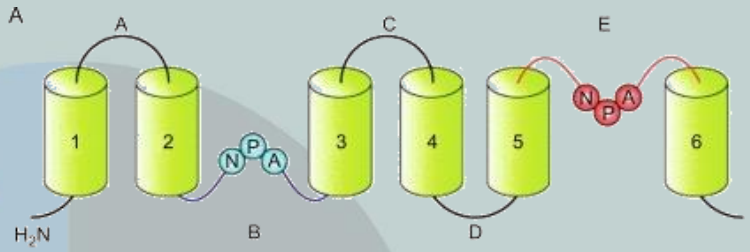
some prokaryotic and eukaryotic cells have water channels, or aquaporin's, embedded in their plasma membrane to allow water to move more rapidly

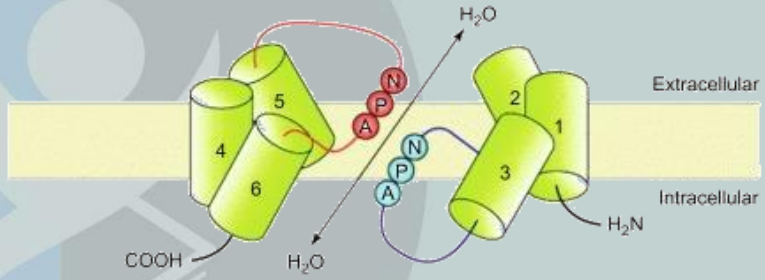
6 α Helix

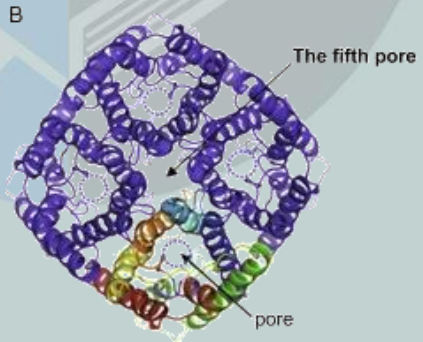
Selectivity filter contain NPA – Sequance

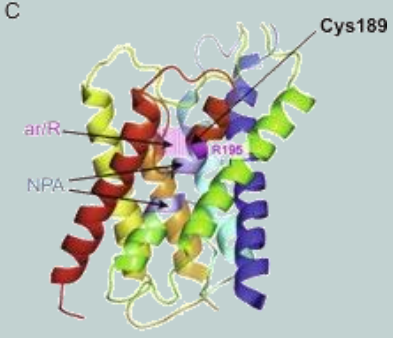
↓

Asn, Pro, Ala.









Aquaporins Are Permeable to Water But Impermeable to Ions

- For most animal cells, however, osmosis has only a minor role in regulating cell volume. This is because most of the cytoplasm is in a gel-like state and resists large changes in its volume in response to changes in osmolarity.

- In addition to the direct difusion of water across the lipid bilayer, some prokaryotic and eukaryotic cells have water channels, or aquaporins, embedded in their plasma membrane to allow water to move more rapidly.

- Aquaporins are particularly abundant in animal cells that must transport water at high rates, such as the epithelial cells of the kidney or exocrine cells that must transport or secrete large volumes of fuids, respectively

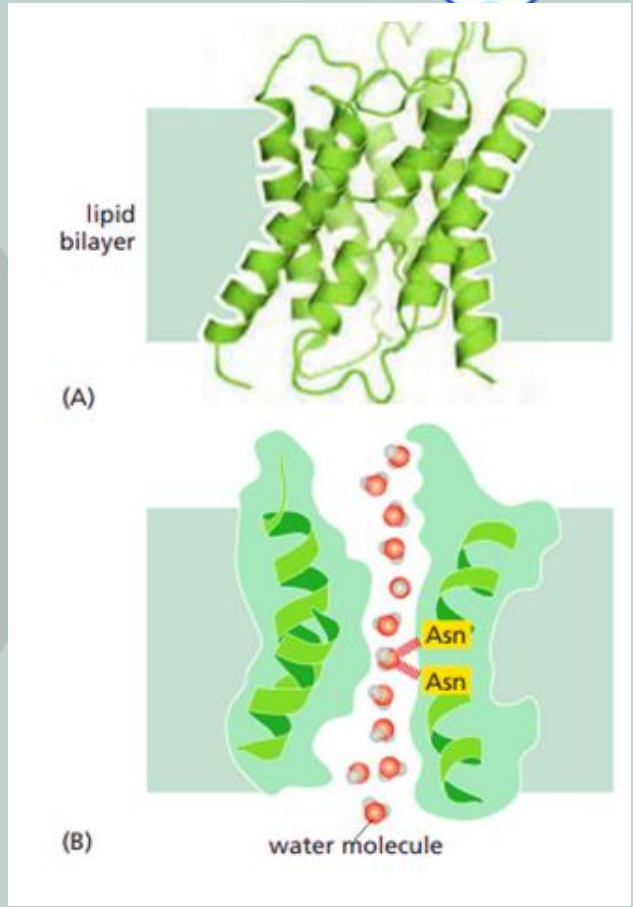
- **The three-dimensional structure of an reveals how it achieves this remarkable selectivity**.

- The channels have a narrow pore that allows water molecules to traverse the membrane in single file, following the path of carbonyl oxygen that line one side of the pore

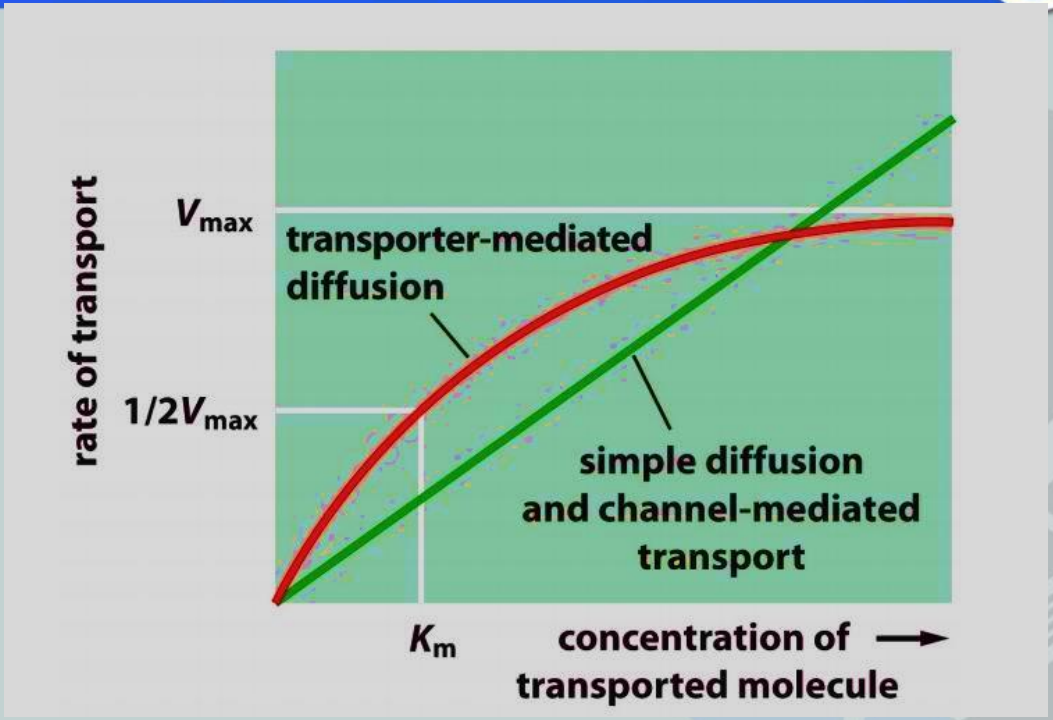
- Hydrophobic amino acids line the other side of the pore.

- The pore is too narrow for any hydrated ion to enter, and the energy cost of dehydrating an ion would be enormous because the hydrophobic wall of the pore cannot interact with a dehydrated ion to compensate for the loss of water.

- impermeable to H+



Diffusion	Facilitated diffusion
Solutes moves from their region of high to low concentration through lipid bilayer	Solutes moves from their region of high to low concentration with the help of transporters
Slow and no saturation	Fast process but saturated when all transporters are occupied
Transport of gases and non-polar molecules	Transport of ions and large polar molecule


rate of transport
V_{max}
$1/2V_{max}$
transporter-mediated diffusion
simple diffusion and channel-mediated transport
K_m
concentration of transported molecule

$$V_{\max} = k_{cat}[E_T]$$

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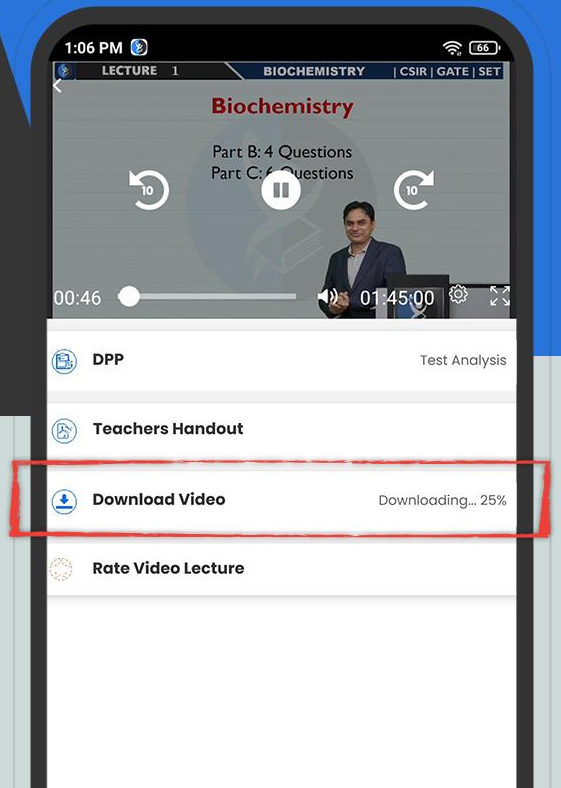

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